El Colegio de la Frontera Sur

Impacto de la florivoría en el éxito reproductivo de una maleza invasora (*Solanum rostratum*)

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Por

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Anexo 9

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Resumen

La florivoría puede afectar el éxito reproductivo de las plantas indirectamente disminuyendo las visitas de los polinizadores y directamente afectando la producción de frutos y semillas. Este estudio evaluó el impacto indirecto de la florivoría sobre el éxito reproductivo a través de la visita de los polinizadores en poblaciones naturales de *Solanum rostratum* Dunal (Solanaceae) en el centro de México. Se caracterizó los florívoros en dos poblaciones y se determinó la preferencia por las estructuras de atracción o reproductivas. Posteriormente, se caracterizó los compuestos volátiles emitidos por flores no dañadas y dañadas por florivoría. Finalmente, se evaluó si el daño (manual y por florívoros) afecta la visita de los polinizadores. Empleando la siguiente metodología, se observaron los florívoros en un cuadrante de 10 m^2 y las estructuras que prefirieron comer, por cinco días. La colecta de volátiles se realizó de flores sin daños y con florivoría, estas últimas expuestas al florívoro por tres horas; mediante microextracción en fase sólida, y la caracterización de los compuestos se efectuó por cromatografía de gases acoplada a espectrometría de masas. Se realizaron bioensayos para determinar la influencia del daño de las estructuras florales sobre la visita de los polinizadores, empleando tres tratamientos: flor sin daño (control), con daño natural y con daño manual. Los principales resultados fueron: identificación de ocho especies de florívoros para esta maleza, que prefirieron mayormente corola, anteras alimenticias y antera polinizadora en ese orden. Identificamos 25 compuestos químicos en proporciones relativas diferentes entre flores sin daños y dañadas. Las flores con florivoría fueron menos visitadas que las flores sin daño. Los visitantes legítimos visitaron más las flores sin daños que los ilegítimos. En conclusión, el daño por florivoría de las flores de *S. rostratum* disminuye el éxito reproductivo por el impacto indirecto a través de la visita de los polinizadores.

Palabras clave: florivoría, florívoros, polinizadores, *Solanum rostratum,* volátiles.

Capítulo I. Introducción

La herbivoría es una interacción planta-animal antagonista en la cual el herbívoro se beneficia mientras que planta se ve perjudicada (Pianka 1978). Esta interacción planta-animal es considerada una de las interacciones más importantes en los ecosistemas debido a la cantidad de biomasa que obtienen los organismos; los herbívoros consumen el 18% de la biomasa terrestre vegetal y el 51% de la biomasa acuática vegetal (Cyr y Pace 1993). Los herbívoros están situados en el centro de las cadenas alimenticias y mantienen el equilibrio o alteran en forma drástica a los niveles tróficos superiores o inferiores a éstos (Granados et al. 2008); debido a que pueden consumir tejidos fotosintéticos, órganos de almacenamiento y estructuras reproductivas (Romero y Vasconcellos-Neto 2007; Del-Claro et al. 2013). Todo lo anterior compromete el crecimiento, la reproducción y la supervivencia de las plantas (Torezan-Silingardi 2007). Por lo tanto, este tipo de interacción es relevante para la dinámica de la población, la ecología de la comunidad y los procesos evolutivos (Malo et al. 2001).

Muchas plantas con flores presentan diversas estrategias para atraer a sus polinizadores; por ejemplo, ofrecen recompensas (néctar y polen) y/o poseen flores atractivas (vistosos colores, olores y formas) (Cardel y Koptur 2010). No obstante, estas flores también pueden ser atractivas para los herbívoros, y consecuentemente ser consumidas por éstos, originándose el fenómeno conocido como florivoría (Burgess 1991; Frame 2003) o herbivoría floral (Cunningham 1995). Las implicaciones de la herbivoría floral sobre el desarrollo y el éxito reproductivo de las plantas han sido poco estudiadas, a pesar de que este tipo de herbivoría puede ocasionar la destrucción de las flores, provocando una marcada disminución en su adecuación (McNaughton 1983; Wallace y O'Dowd 1989; Lowenberg 1994; Cunningham 1995; Ferreira y Torezan-Silingardi 2013). Además, resulta sorprendente la poca información en este tema si se considera que, la evolución y radiación de las plantas con flores y los insectos están relacionadas con la alimentación oportunista y obligatoria de las flores y las estructuras reproductivas (Ehrlich y Raven 1964). Así, los florívoros pueden desempeñar un papel importante en la evolución de los rasgos florales en las plantas (Frame 2003).

La florivoría puede afectar el éxito reproductivo de las plantas a través de dos mecanismos: a) directamente por el consumo de los florívoros de todos o parte de los órganos reproductivos (pistilos y estambres), así como el consumo de gametos (polen y óvulos) afectando el éxito reproductivo masculino y femenino (Muenchow y Delesalle 1992; Krupnick y Weis 1999; Leege y Wolfe 2002; Canela y Sazima 2003); o b) indirectamente por medio de la alteración del atractivo de las flores para atraer a los polinizadores (Karban y Strauss 1993; Cunningham 1995; Lohman et al. 1996; Breadmore y Kirk 1998; Krupnick et al. 1999; Mothershead y Marquis 2000) afectando diversos caracteres florales incluyendo el tamaño del pétalo (Mothershead y Marquis 2000), la producción de néctar (Krupnick et al. 1999) y la producción de flores o inflorescencias (Lohman et al. 1996). También se puede afectar la cantidad y calidad de las flores dañadas o de las flores producidas posteriormente (Mothershead y Marquis 2000, McCall 2006); así como la cantidad y calidad de las visitas de los polinizadores, que pueden interactuar de forma aditiva o no aditiva para afectar la adecuación de la planta. Además, la polinización puede alterarse a través de la competencia con otros polinizadores (Canela y Sazima 2003) o afectando el suministro de polen de toda la población (Bertness y Shumway 1992). El efecto de la florivoría en el éxito reproductivo depende de diversos factores, incluyendo el tipo y la cantidad de daño floral, sistema de apareamiento y rasgos de la historia de vida de las plantas, así como las interacciones antagonistas y mutualistas en la comunidad (McCall e Irwin 2006).

La florivoría también puede afectar las interacciones con los polinizadores al alterar el contenido y la composición de los atrayentes florales. Los florívoros pueden liberar componentes químicos cuando dañan el tejido y pueden contribuir con olores extraños al producir productos de desecho alterando los aromas florales que atraen a los polinizadores (Zangerl y Berenbaum 2009). Se conoce que los volátiles florales actúan como sinomonas (sustancias químicas emitidas por un organismo, que al ponerse en contacto con individuos de otras especies, provoca en el receptor una reacción de comportamiento o fisiológica adaptativamente favorable para ambas especies (Nordlund y Lewis 1976) para los organismos mutualistas, i.e., polinizadores (Faegri y van der Pijl 1979, Tan y Nishida 1998, 2000, 2007). Sin embargo, algunas de estas mismas sustancias pueden funcionar como kairomonas (mensajeros químicos interespecíficos cuyos beneficios adaptativos recaen en el organismo receptor de la señal química en lugar del emisor (Brown et al. 1970) para los antagonistas. Por tanto, las plantas que anuncian la disponibilidad floral puede aumentar al mismo tiempo la probabilidad de ser detectadas por los florívoros como sucede en *Cirsium arvense* (Theis 2006, 2007; Andrews et al. 2007)

Finalmente, la herbivoría genera costos directos para las plantas cuando los recursos son limitados, especialmente en los casos en que las plantas reabsorben nitrógeno y fósforo de los pétalos una vez que se ha producido la fertilización (Ashman 1994) o cuando las estructuras florales y los ovarios se consumen a finales de la temporada de floración, una vez que se han comprometido recursos para desarrollar estructuras reproductivas y embriones (Lowenberg 1994). Por lo tanto, la destrucción o reducción del área floral en estas especies de plantas, puede reducir la cantidad de carbono disponible para la asimilación en la maduración de frutos y semillas. Además, los pétalos dañados pueden perder agua debido a que se incremente la pérdida transpiracional o simplemente a través de fugas (Galen et al. 1999). Así como, la florivoría podría aumentar el estrés hídrico en las flores dañadas especialmente en ambientes secos (Galen et al. 1999). Las consecuencias directas de la florivoría que provocan la disminución de la reabsorción de nutrientes, la reducción de la fotosíntesis o cambios en la pérdida transpiracional han sido escasamente estudiados (McCall e Irwin 2006).

De igual manera los estudios que analizan el impacto de la florivoría sobre la polinización de plantas con morfología floral especializadas son escasos. En este estudio se documenta cómo dicha interacción (planta-florívoro) impacta en la visita de los polinizadores; utilizando como sistema de estudio a *Solanum rostratum* Dunal (Solanaceae) debido a que constituye un excelente modelo por su compleja morfología floral (dimorfismo en las anteras y polimorfismo floral) y su estrecha relación con los polinizadores. Además, sus flores ofrecen polen como única recompensa y emiten una fragancia floral (Bowers 1975) semejante al aroma del durazno por lo que se le conoce comúnmente como "duraznillo" (Nee 1993). Las anteras son las responsables de la emisión de los compuestos volátiles (Solís-Montero et al. 2018). Esta maleza es probablemente nativa de México (Whalen 1979) pero se ha extendido como especie invasora a Estados Unidos, Canadá y Argentina (Bassett y Munro 1985; Del Vitto y Petenatti 2015), incluso a otros continentes como Asia, Europa y Australia (Whalen

1979; Zhao et al. 2013). Además, esta maleza es de interés agrícola, debido a que es hospedera de plagas que afectan a las plantas cultivadas, no sólo en su rango introducido, sino también en el rango nativo. Por ejemplo, es hospedera natural del escarabajo colorado de la papa (*Leptinotarsa decemlineata* (Say)) que es una plaga de importancia económica para la papa, *Solanum tuberosum* L. (Brues 1940; Rodríguez-Islas 2016). Además, de producir solanina en sus hojas y frutos, siendo tóxicos para el ganado y puede contaminar el grano de cereal (Parsons y Cuthbertson 2001). Evaluar el efecto de la florivoría en el éxito reproductivo de *S. rostratum* es relevante para comprender la importancia de los florívoros que dañan las poblaciones naturales de esta maleza, ampliando el conocimiento en el campo del control biológico de esta especie.

Does florivory affect the floral visitors' attraction of a buzz- pollinated herb?

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Abstract

Floral herbivory (florivory) can affect directly and indirectly plant reproduction through ovules or seeds lost or by reduction in flower attractiveness to decreasing pollinators' visitation, respectively. In this work, we studied the effect of florivory on reduction of pollinator's visitation in a buzz-pollination herb. We used as study model *Solanum rostratum* because its heterantherous flowers produce a floral scent that suggests a close association with its pollinators. We hypothesized that when florivorous consume the reproductive structures (anthers or pistyle) or the attraction structures (corolla) the pollinator's visitation decrease affecting *S. rostratum* reproduction. We conducted observation in two populations of central Mexico. We observed eight species of florivorous consuming *S. rostratum* flowers. Florivorous preferred to consume attraction structures (corolla) to reproductive structures. However, they preferred to consume the anthers specialized in feeding functions to the anthers specialized in pollination. We recorded floral volatiles emitted by damaged flowers (florivory) using solid phasemicroextraction coupled with gas chromatography-mass spectrometry. We identified 25 volatile compounds in *S. rostratum* flowers, mainly aromatic, monoterpene and sesquiterpene compounds. The proportion ratio of these compounds differed between undamaged and damaged flowers. Bioassays showed that legitimate visitors (pollinators) and illegitimate visitors (thieves) visited more often undamaged flowers than damaged flowers by florivorous. In conclusion, the consumption of floral structures (attraction and reproduction) decreases pollinator visitation; suggesting that this probably affect *S. rostratum* reproduction.

Keywords: Buzz-pollination, florivory, pollinators, reproduction, *Solanum rostratum.*

Introduction

Mutualistic pollinators fertilize most of the flowering plants; however, at the same time antagonistic visitors (florivorous, folivorous, and seed predators) attack these plants (Liao et al. 2013). The herbivorous attack plants at different times of their growth and development (Kelly et al. 2008; Penet et al. 2009). Florivorous focus to damage bracts, sepals, petals, stamens and pistils, as well as pollen and ovules of flower buds or mature flowers (Burgess 1991; Frame 2003). Some of these herbivores also consume photosynthetic tissues and storage organs (Romero and Vasconcellos-Neto 2007; Del-Claro et al. 2013). This damage can compromise the growth, reproduction and survival of plants (Torezan-Silingardi 2011).

Consumption of floral structures or florivory negatively affects pollination (Krupnick et al. 1999; McCall and Irwin 2006; Sánchez-Lafuente 2007; Cardel and Koptur 2010), either by destroying the internal structures of the buds and/or by reducing the attractiveness of flowers to pollinators (Lowenberg 1994). Florivorous that feed on male and female gametes (pollen and ovules, respectively) directly reduce fertilization success. In addition, damage to corollas and calyxes, which function is attracted pollinators, may indirectly decrease reproductive output due to a reduction on pollination services (Krupnick et al. 1999; Sánchez-Lafuente 2007; Cardel and Koptur 2010). These direct and indirect effects of florivory on the reproductive fitness of plants can be important in the adaptive evolution of flower traits and reproductive systems of flowering plants (Frame 2003; McCall and Irwin 2006; Bartkowska and Johnston 2012).

Florivory is a widespread phenomenon in flowering plants but relatively few studies are known (Breadmore and Kirk 1998; McCall and Irwin 2006). Particularly, studies that evaluate the impact of florivory on reproductive success of plants with specialized pollination are scarce. Therefore, we used *Solanum rostratum* Dunal (Solanaceae) as a study system because this species is an excellent model due to its complex floral morphology and the close relationship with its pollinators. In addition, this herb offers pollen as the only reward and emits a floral fragrance similar to peach odor (Bowers 1975; Nee 1993) by its poricidal anthers (Solís-Montero et al. 2018). Moreover, this herb is pollinated by bees which extract large quantities of pollen using vibrations (i.e. buzzing) (Buchmann and Hurley 1978; Buchmann 1983). These buzzing bees are large and medium size, which make contact with sexual organs during visitation, in its native and invasive range of distribution (Solís-Montero et al. 2015; Zhang and Luo 2008). This weed is probably native to Mexico (Whalen 1979); although, it has spread as an invasive plant to the United States of America, Canada and Argentina (Bassett and Munro 1985; Del-Vitto and Petenatti 2015), even to other continents such as Asia, Europe and Australia (Whalen 1979; Zhao et al. 2013).

Moreover, this weed is a host of pests that affect cultivated plants in their native and introduced range of distribution. For example, it is a natural host of the Colorado potato beetle (*Leptinotarsa decemlineata* (Say)), which is an important pest of potato plants *Solanum tuberosum* L. (Brues 1940; Rodríguez-Islas 2016). In addition, this plant produces solanine in its leaves and fruits that is toxic to livestock and can contaminate cereal grains (Parsons and Cuthbertson 2001).

We are interested in knowing whether florivorous decrease the pollination attraction of natural populations of *Solanum rostratum*. We hypothesized that when florivorous consuming the reproductive structures (anthers or pistyle) or attraction structures (corolla) decrease pollination visitation affecting *S. rostratum* reproduction. Thus, we aimed to answer the following questions: a) Which insects consume the flowers of *S. rostratum* in two native populations?; b) Do florivorous prefer to consume reproductive or attraction structure of the flowers of *S. rostratum*?; c) Does chemical profile of floral volatiles differed between damaged flowers by florivory and undamaged flowers?; d) Do pollinators attraction is affected by florivory?

Materials and methods

Studied species

The flowers of *S. rostratum* are heterantherous, *i.e.*, each flower produces two types of anthers: four anthers specialized in feeding function and one anther specialized in fertilization (Vallejo-Marín et al. 2009; Vallejo-Marín et al. 2010; Zhao et al. 2013). The feeding anthers (FA) are bright yellow and are located in the center of the flowers that mainly attract pollinators to visit these flowers. On the other hand, the pollinating anther (PA) is a yellow-brownish anther deflected to the right or left side of the flower opposite to the style. This larger anther contributes disproportionately to fertilization (Vallejo-Marín et al. 2009). Each plant produces right- and left-handed enantiostylous flowers in equal proportions (Jesson et al. 2003). There is not apparently difference in pollen fertility between the two types of anthers or the two types of flowers in *S. rostratum* (Bowers 1975). However, there is difference in volatile compounds proportions between anther types (Solís-Montero et al. 2018).

Characterization of florivorous in populations of *S. rostratum*

We characterized the florivory of two populations of *S. rostratum* in Central Mexico (Puebla State). The first population was located in Amalucan hill (19°02'52.6''N, 98°07'59.4''W, 2234 m asl) near an urban area. The second population was located in San Andrés Cholula (19°02'53.6''N, 98°17'13.5''W, 2136 m asl) more exposed to anthropic activity than first population, surrounded by an urban area. In order to observe the florivorous in both populations, we used the Rodríguez-Islas (2016) methodology referred to the evaluation of the impact of florivory on reproductive success, directly and indirectly. The florivorous observations were conducted on 29th June and 5th, 6th, 9th and 16th July at Amalucan population and at Cholula population from 16th to 21th of August 2018.

Each day of observation, a quadrant of 2.5 x 2.5 meters was observed. The flowers open 30 min after sunrise and remain open until the afternoon (07:30-17:00 h CDT; Bowers 1975). Therefore, the observation was conducted when the flowers were opened, in periods of 30 min of observation with intervals of 1 h between each period during 5 days. We recorded the following data: a) identity of the florivorous (species or morphospecies), b) part of flower consumed (feeding anthers, pollinating anther or corolla), c) floral display (number of flowers open per quadrant) and d) duration of florivorous consumed the floral structures. At the end of observation, the florivorous were stored in alcohol 70% for taxonomical identification. Voucher specimens were deposited in the entomological collection of El Colegio de la Frontera Sur (ECOSUR), Tapachula Campus, Chiapas, Mexico.

Volatile compounds emitted after florivory of *S. rostratum* flowers

Volatile sampling

We sowed seeds in the greenhouse from a wild population of the Amalucan hill, Puebla, Mexico previously collected (5 fruits per individual, 15 plants) in 2013. The seeds were stored in paper bag at 4°C until planting. One hundred and fifty seeds were planted in ECOSUR greenhouse according Vallejo-Marín et al. (2013) methodology. After plants started to bloom, the samples were collected for 14 plants chose randomly during seven days (3rd, 5th, 6th, 7th, 9th, 10th and 12th April 2018). For each plant, we sampled two undamaged flowers (control) and two damaged flowers by florivory (28 flowers per treatment). The florivory was caused by specimens of the genus *Lethus* sp. (Orthoptera) using a clip cage [cage of foam tube (4 cm height x 4 cm inner diameter) covered with fine tulle on both sides of the tube]. We captured the specimen were maintained in transparent plastic box (34.6 cm length x 21 cm width) cover with fine. The next day, they were placed for three hours (from 07:00 to 10:00 h) on an undamaged flower in a clip cage so florivorous can consume the different floral structures of flower.

We chose an orthopteran because it was the most abundant florivorous in field and they consumed the floral structures faster. The flowers were exposed to grasshopper for three hours (07:00-10:00 h); previously the grasshopper was left for 24 h fasting. The volatile sampling was performed out from 10:00-16:00 h using of solid phase microextraction devices (SPME) fitted with fibers coated with 65 µm polydimethylsiloxane-divinylbenzene (PDMS-DVV, Supelco, Belfonte, PA, USA). We considered this period because we recorded the highest pollinator activity and the strongest peach odor release (from 10:00-12:00 h) in the greenhouse. After florivorous were removed, the two flowers of each treatment (control and florivory flowers) per plant were placed inside a glass container (14 cm height x 2.5 cm diameter), and the flower headspace was sampled during 6 h. After the period sampling, the fiber was withdrawn and inserted into the injector of gas chromatograph-mass spectrometer (GC-MS). The samples were desorbed for 1 min in the GC injector for analysis.

Characterization of volatile compounds of flowers with florivory

Volatiles emitted by flowers were analyzed with a GC Varian Model CP-3800 equipped with a non-polar capillary DB5-MS column (30 cm by 0.25 mm ID, and 25 µm coat thickness) coupled to a Varian Saturn 4D mass spectrophotometer with integrated data system. Helium was used as a carrier gas. The samples were analyzed by an initial temperature program of 50° C for 2 min with an increase of 15° C/min up to a temperature of 280° C for 10 min. The temperature of the injector was 250° C. The ionization was performed by electronic impact at 70 eV and 250° C.

Compounds were tentatively identified by comparing their retention index and mass spectra with those in the mass spectra library (NIST library version 2.5, National Institute of Standards and Technology). Identification of some compounds was confirmed by the comparison of mass spectra and retention times with those of standards. The standards were purchased from Sigma-Aldrich (Toluca, Mexico) and were 97-99% pure according to the supplier. The relative abundance of a particular compound was calculated as the proportion of its area to all GC areas combined. Later, we calculated the mean relative abundance for each compound in both treatments (damaged or undamaged flowers).

Effect of florivory on floral visitors and seed set

We carried out bioassays in field conditions to determine the impact of florivory on floral visitors' attraction. In order to determine the impact on visitation rate (legitimate and illegitimate visitors) caused by the damage of the reproductive structures by florivorous, we conducted the following three treatments: a) flowers with mechanical damage: 50% of both anthers and of the corolla were eliminated using a small scissor; b) flowers with natural damage: we placed *S. rostratum* flowers with an orthopteran (family Acrididae) in a chamber (clip cage) during 3 h. We selected an orthopteran because this insect was abundant in the population and consumed *S. rostratum* flowers (see results). The specimens were previously collected in the field and moved in container jars without food for 24 h. Finally, c) we used as control undamaged flowers. Four plants were selected at random per day, each plant contained the three treatments and two replicates per treatment (6 flowers per plant) tagged individually. No damage was observed in leaves. Pollinator observations were conducted only in tagged flowers during the peak of visits (10:00-12:00 h). The observation period was conducted for 20 min and 5 min between periods. In each repetition, we observed by flower: 1) the number of visits, 2) the duration of each visit, 3) the identity of visitors (species or morphospecies), and, 4) whether floral visitor contacted the sexual organs. We collected 4 specimens of each type of visitor to taxonomical identification. Voucher specimens were deposited in the Universidad de Las Américas Puebla (UDLAP) collection. The bioassays were conducted for 8 days (30^{th} June, 10^{th}, 13^{th}, 17th, 18^{th}, 24^{th}, 25^{th} and 27^{th} July). After 6 weeks, the fruits were collected and transported to ECOSUR to quantify the seed production in laboratory.

Statistical analysis

We used statistic descriptive in the number of visits of the florivorous according to taxonomic groups, the abundance of the florivorous by periods of observation, the visits of legitimate and illegitimate visitors to the different treatments. A Kruskal-Wallis test was used to know whether visitation rate and the visits time (i.e., time that florivorous spend consuming floral structure) differ among treatments (manual damage, florivory or control).

When we found statistical differences, we conducted a Dunn test using Bonferroni correction with FSA package (Ogle 2017). The relative abundance of volatile compounds in each sample was compared via Principal Components Analysis (PCA). We used an analysis of variance (ANOVA) of PC scores to test whether the relative abundance of compounds was different between undamaged and damaged flowers differed. For all analysis, we used the statistical package R v.3.5.1 (R Core Development Team 2018).

Results

Florivorous in populations of *Solanum rostratum*

We found eight species of florivorous corresponding to two orders and to four families of insects in the populations of Amalucan and Cholula (Table 1). The florivorous which consumed more flowers from both populations were: *Epitrix* sp.; followed by Sp.1 and *Centralaphthona* sp. in Cholula population and Sp.3 and Sp.2 in Amalucan population (Fig. 1). In Amalucan population, *Epitrix* sp. was the most abundant species that carried out 27% of visits, with a peak between 15:00-15:30 h. On the other hand, in Cholula population this species performed 50% of visits but majority of them were recorded earlier from 13:30-14:00 h. The Sp.1 in Amalucan recorded 13% of visits which highest number of visits was carried out from 13:30-14:00 h. Meanwhile, in Cholula Sp.1 performed 22% of the visits, with a peak between 10:30-11:00 h. The Sp.3 had a higher number of visits in Amalucan (20%) than in Cholula (3%) population. The Sp.2 was found exclusively in Amalucan population (Table 1, Fig. 1).

The florivorous preferred to consume the corolla (>69 % of visits) in both populations, and they consumed less frequented the stigma (< 0.8 % of visits). In general, florivorous consumed more the feeding anthers (14 and 17% of visits) than pollinating anther (9 and 12%) in both populations, except for *Macrodactylus mexicanus* and Sp.1 in Amalucan population, and *Epitrix* sp. in Cholula population (Table 2). We found that time of florivorous spent consuming floral structures did not differed among florivorous type in Amalucan population (122.54 ± 84.28 s) (Kruskal-Wallis test, X^2 = 0.3859, DF = 2, P = 0.8245) neither in Cholula population (97.09 ± 18.11 s) ($F_{2,9}$ = 0.041, P = 0.96). In addition, the visitation rate was not different between Amalucan population (Kruskal-Wallis test, X^2 = 6.0108, DF = 5, P = 0.3052) and Cholula population (Kruskal-Wallis test, X^2 = 5.7328, DF = 4, P = 0.22).

Chemical analysis of compounds in flowers in damage of *S. rostratum*

We found 25 chemical compounds in undamaged and damaged flowers by florivorous, including alkanes, alkenes, terpenes, sesquiterpenes, aromatics, alcohols, aldehydes and esters. The chemical composition of the volatile compounds differed quantitatively between the undamaged and damaged flowers (Table 3). The volatile compounds found in major abundance in both damaged and undamaged flowers were α-copaene, methyleugenol, unknown 2 compound and γ-decalactona (Table 3).

The PCA of the chemical composition of volatiles emitted by undamaged flowers and damaged flowers showed that two main components accounted for 50% of the variance. The first principal component (PC1) explained 28% of variance while the second component (PC2) explained 22% of variance. The PC1 is mainly explained by α-copaene negative relate with nine compounds (see bold numbers of Table 4) which reported the highest values; however, the PC1 was not different between undamaged and damaged flowers ($F_{1, 12}$ = 1.1522, P = 0.7033). The α-copaene was the compound found in major proportion in both types of flowers. This compound clearly increased its proportion in damaged flowers (Table 3). On the other hand, the PC2 is main explained by δ-cadinene which is positive related with other compounds such as γ-cadinene, β-cadinene, γ-decalactona, γ-muurolene, β-sinensal, methyleugenol and

(Z)-2- tridecene, but all these compounds were negative related with α-copaene. The PC2 differed between undamaged and damaged flowers ($F_{1, 12}$ = 4.986, P = 0.04537) (Fig. 2; Table 4).

Effect of florivory on floral visitors and seed set

The main visitors of *S. rostratum* were bees (Hymenoptera: Apoidea and Halictidae); however, some hover flies Diptera: Syrphidae) also visited the flowers. The duration of visits differed between species. *Xylocopa* sp. and *Bombus sonorus* spent few seconds (3 and 5 s, respectively) per visit in comparison with other bee species that spent more time (6-10 s) per visit. Although, we observed that the duration of visits of *Antophora* and *Apis mellifera* was a short time (4 s) per visit. Floral visitors such as *Bombus sonorous* and *Xylocopa* sp. were classified as legitimate visitors because they contacted both sexual organs (the stigma and anthers) in the majority of visits (> 66%). *B. sonorous* visited more flowers of *S. rostratum* compared to other species (Table 5). The remaining taxa were considered as illegitimate visitors because they few times contact the stigma (< 28%) or never contact it. However, illegitimated visitors often contacted anthers (47%). In general, both type of visitors visited more the feeding anthers (21%) than the pollinating anthers (10%) (Table 5).

The number of visits according to treatment (flowers damaged by florivorous or manually and undamaged flowers) did not differ between type of visitor (Z = -0.663, P = 0.5). For that reason, we decided to analyze all data together. The results reveal that damaged flowers by florivorous received less visits than undamaged flowers (Fig. 3A) conducted by floral visitors (legitimate and illegitimate). However, flowers damaged manually did not differ from undamaged flowers neither from damaged flowers by florivorous (X^2 = 11.303, DF = 2, P = 0.003) (Fig. 3A). We found that the duration of visits was lower in flowers damaged by florivory than those undamaged. Moreover, the duration of visits from the flowers manually damage did not differ from undamaged or damaged flowers by florivorous (X^2 = 11.333, DF = 2, P = 0.003) (Fig. 3B). We found that fruit and seed set differed among treatments: damaged flowers by florivorous (30% fruit set; 59.83 ± 8.6 seed), damaged flowers manually (40% fruit set; 58.13 ± 12.2 seeds) and undamaged flowers (50% fruit set; 64.80 ± 9.1 seeds); however, these differences were not statistically significant (fruit set: X^2 = 2, DF = 2, P = 0.3679; seed set: X^2 = 1.8255, DF = 2, P = 0.4014).

Discussion

The floral herbivory is the damage cause by animal to the reproductive structures (anthers or pistil) or attractive structures of flowers (i.e., petals, sepals, bract or ligules) (Burgess 1991; Karban and Strauss 1993; Krupnick and Weis 1999; Adler 2000; Mothershead and Marquis 2000; McCall and Irwin 2006; Cardel and Koptur 2010; McCall 2010; Botto-Mahan et al. 2011). However, floral consumption is a widespread phenomenon in flowering plants, florivory and its repercussion over development and reproductive success have been little studied, even though it affect plant reproduction due to damage cause in flowers (McNaughton 1983; Wallace and O'Dowd 1989; Lowenberg 1994; Cunningham 1995; Breadmore and Kirk 1998; McCall and Irwin 2006). For example, in one of the largest genus of flowering plant, *Solanum*, which contains approximately 1400-1700 species (Frodin 2004; Bohs 2005) some of them with agricultural importance, few studies explore this antagonistic interaction (Bohs and Olmstead 1999; Frodin 2004; Knapp et al. 2004; Bohs 2005; De Luca et al. 2013; Vallejo-Marín et al. 2013). Wise and Herbet (2010) conducted one of these few studies, they reported in *Solanum carolinense* four florivorous (*Anthonomus nigrinus, Leptinotarsa juncta, Microtus pennsylvanicus* and *Frumenta nundinella*) two coleopterans, one mammal and a lepidopteran, respectively. In our study, we found 8 species of florivorous consumed *S. rostratum* flowers, all of florivorous were insects from two orders (Coleoptera and Orthoptera) and four families (Coleoptera: Chrysomelidae, Scarabaeidae and Melyridae; Orthoptera: Acrididade); the two first families of Coleoptera were previously reported by Rodríguez-Islas (2016) in other population of *S. rostratum* in Mexico. We believed that it is relevant to study the antagonists associated with this weed for two reasons: 1) to expand the knowledge of antagonist interactions would probably regulated populations of this weed, which is widespread as invasive plant around the world. 2) Because this herb could be reservoir of pests of other solanaceous cultivated plants such as *L. decemlineata* not only in its native range of distribution but also in its invasive range (Brues 1940; Rodríguez-Islas 2016).

Recently, the ecological effects of florivory have begun to be examined (McCall 2008; Hanley et al. 2009; Penet et al. 2009); however, there are few studies to explain how florivorous choose flowers for consumption (see Bandeili and Müller 2010). According to McCall and Irwin (2006), florivorous preferences could play an important role in floral trait selection such as shape or size of corollas and chemical defences in nectar or other floral parts. For example, Galen and Cuba (2001) found in *Polemonium viscosum* that flowers with wider corollas were attacked more than those with narrower corollas. Other example, larger flowers of *Cistus ladanifer* received more floral damage by ants than smaller flowers (Teixido et al. 2011). Another example, McCall and Barr (2012) found that corolla diameter explained the florivorous preference more than anther presence or corolla color. This last study corroborated our findings that florivorous preferred to consume corolla (69% of visits) to anthers. However, florivorous preferred to consume feeding anthers (17% of visits) to pollinating anthers (12 % of visits). This last result could be explained because feeding anthers are preferentially visited by bees for pollen consumption due to visual and olfactory cues (Li et al. 2015; Mesquita-Neto et al. 2017; Carvalho Velloso et al. 2018; Solís-Montero et al. 2018) which probably also attack florivorous.

Therefore, the effect of florivory can reduce flower attractiveness by altering various floral traits such as petal size or nectar production reducing pollination visits (Lohman et al. 1996; Krupnick et al. 1999; Mothershead and Marquis 2000; McCall and Irwin 2006; Sánchez-Lafuente 2007; McCall 2008; Cardel and Koptur 2010; Parra-Tabla and Herrera 2010). For example, flowers of *Nemophila menziesii* with simulated florivory presented a decrease on pollinator's visitation on artificial (McCall 2008) and natural conditions (Lohman et al. 1996). According to Tsuji and Ohgushi (2018) the decrease of pollinator visit rate could be related to corolla consumption. These examples show that florivorous affect the pollination visitation, which often reduce plant reproductive output through fruit and seed production (Lowenberg 1997; Krupnick and Weis 1998; Kessler et al. 2011). In our study, we clearly demonstrated that florivory reduced floral visits not only conducted by legitimate visitors (pollinators) but also conducted by illegitimated visitors (thieves) in a buzzed-pollinated herb. The legitimated visitors, *B. sonorous* and *Xylocopa* sp., were previously reported as effective pollinators of *S. rostratum* because these large buzzing-bees contact sexual organs when visited the flowers (Bowers 1975; Solís-Montero et al. 2015) as this study corroborated.

In this study, we found that fruit and seed production was higher in undamage than in damaged flowers by florivory; however, these differences were not statistically significant probably because we need to increase the number of samples. Indeed, some studies show that florivorous reduces the amount of fruits in plants; in *Opuntia macrocentra* the amount of fruit was reduced between 20% and 100% by the consumption of floral structures by the larva of *Olycella subumbrella* (Mandujano et al. 2013). *Olycella junctolineella* reduced fruit set in *O. microdasys* by up to 100% in plants with much florivory and on average was around 21% in flowers less damaged (Piña et al. 2010). Other species with florivory reduce fruit set from 55% to 64% (Sánchez-Lafuente 2007; Martínez-Peralta and Mandujano 2011).

It is know that *Solanum rostratum* strongly depend on pollinators to reproduce, when pollinators are scare pollen-limited fruit and seed production (Solís-Montero et al. 2015). However, in this study did not found difference on fruit or seed production among damage and undamaged flowers, we attributed to flowers were not previously excluded before experiment and low number of fruit sampled. Some studies suggest that florivory decreases fruit and seed set indirectly via decreasing pollinator attraction (Pellmyr and Thompson 1996; Le Corff et al 1998; Ohashi and Yahara 1998; Parra-Tabla and Bullock 1998; Bigger 1999; Krupnick and Weis 1998; Strauss and Agrawal 1999; Strickler and Freitas 1999; Cunningham 2000; McCall and Irwin 2006; Strauss and Whittall 2006; Tsuji and Ohgushi 2018). Then it is necessary in future research to conducted bioassays with more controlling conditions to probe the effect of reduction of visitation rate in fruit and seed production in *S. rostratum*.

Our results also show that florivory reduce pollen theft, which is very common in natural population of *S. rostratum* (Solís-Montero et al. 2015). The pollen thieves reported in this study were *Augochlora sp.*, *Augocloropsis metallica*, and *Lasioglossum dialictus* previously reported in other populations of *S. rostratum* (Solís-Montero et al. 2015); these small buzzing-bees precluded contacting with sexual organs and visiting most of the times only the anthers. On the other hand, *Anthophora* sp., medium buzzing-bee, was previously reported as floral visitor of *S. rostratum* in non-native population (Bowers 1975)*;* however, in this study we demonstrated that this bees acted as conditional thieves or poor pollinator because represent the minimum in continuum of pollinator efficiency (Hargreaves et al. 2009); only 16 % of the visits conducted were legitimate visits (touch stigma). *Apis mellifera,* medium non-buzzing bee, is also considered conditional thieves (Solís-Montero et al. 2015) conducted 28% of legitimate visit in this study. Pollen larceny can affect plant fitness directly by reducing pollen for fertilization or indirectly by reducing attractiveness of robbed flowers (Hergreaves et al. 2009). This study show that also pollen thieves preferred to visit undamaged flowers than damaged flowers by florivory. This result suggests that florivorous could focus to theft undamaged flowers indirectly affecting pollinator attractiveness. It is necessary in future research the study the impact of interaction florivore-plant-pollinator in plant reproduction.

Our results suggest that florivory reduces the pollinators and thieves visitation to flowers. This could be attribute to several reasons: alteration of the attractiveness of flowers (Karban and Strauss 1993; Cunningham 1995; Lohman et al. 1996; Breadmore and Kirk 1998; Krupnick et al. 1999; Mothershead and Marquis 2000) due to reward levels (Krupnick et al. 1999) or floral morphology modifications (Kudoh and Whigham 1998; Sánchez-Lafuente 2007; McCall 2008), and/or changes in floral volatile composition (Theis et al. 2007; Zangerl and Berenbaum 2009). The last reason imply that modification in volatile floral compounds emission in response to florivorous can reduce attractiveness for pollinators (Theis et al. 2007; Zangerl and Berenbaum 2009; Dicke and Baldwin 2010; Junker et al. 2010). However, even to date the effects of florivory on the volatile profile of flowers have not been extensively investigated (Zangerl and Berenbaum 2009; Muhlemann et al. 2014). The flowers produce bouquets of aromas that together with the visual signal attract the pollinators (Raguso 2008); although, it can increase the probability that plants also attract herbivores (Hartmann 2008) then floral aromas can also act as defensive from these antagonists (Strauss and Whitthall 2006). In this study, we found that the main difference between undamaged and damaged flowers was the proportion of the major floral volatile compounds, which were mainly aromatic (e.g., methyleugenol), monoterpene (e.g., terpinolene) and sesquiterpene (e.g., α-copaene) compounds corresponding to floral VOCs (Schiestl 2010). We found higher proportions of methyl eugenol in undamaged flowers and α-copaene in damaged flowers. Previous studies reported that the behaviour of the arthropods responds to the influence of the different proportions of the compounds. For example, methyl eugenol strongly attracts several pollinators (especially flies and bees, Dobson 1994; Tan and Nishida 2012); whereas when this compound is presented at high concentrations repels herbivores (Tan and Nishida 2012). The α-copaene at low concentrations strongly attracts flies (*Ceratitis capitata*) but a high doses flies exhibited abnormal behaviour (Nishida et al. 2000). Beck et al. (2015) found that α-copaene with other compounds were identified as a probable volatile biomarker indicating damage to the floral tissue. It is necessary research the possible role α-copaene as semiochemical for floral herbivores. In contrast, γ-decalactone was detected in higher proportion in undamaged flower than in damaged of *S. rostratum*. This compound is uncommon compound in plants (Knudsen et al. 2006) but it is responsible for peach odour in fruits (Zhou et al. 2018), there is not information regarding its role in bee foraging behaviour (Solís-Montero et al. 2018). It is necessary in future studies to elucidate its role on pollination or florivorous attraction. The response of plants to damage by herbivores is to release several volatile substances, whose profile markedly differentiated between undamaged or mechanically damaged plants (Paré and Tumlinson 1999) which differed in quantitative and qualitative emission pattern (Schaub et al. 2010). Volatiles proportion changes between damage and undamaged could be one of the reason that explain why floral visitors in this study made more visits to the undamaged flower.

In conclusion, our study demonstrated that florivory reduced the visitation of floral visitors (pollinators and thieves). This is probably explained by damage produce by florivorous mainly on attractive (corolla) and reward structures (feeding anthers). In addition, it could be explain by changes in proportion of floral volatiles compounds in damaged flowers. This suggests that visual and chemical cues modifications cause by florivorous could play an important role on pollinators and pollen thieves reduction on flower visitation in a buzz- pollinated plant.

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Table 1 Percentage of visits (number of visits) in two populations of *Solanum rostratum* in Puebla, Mexico

Florivorous			% Number of visits	
Orden	Family	Species	Amalucan	Cholula
Coleoptera	Chrysomelidae	*Diabrotica undecimpuctata duodecimnotata*	0	3.21 (7)
		Epitrix sp.	26.67 (68)	50.00 (109)
		Centralaphthona sp.	3.53 (9)	21.10 (46)
	Scarabaeidae	*Macrodactylus mexicanus*	8.24 (21)	0
	Melyridae	Sp.1	12.55 (32)	22.48 (49)
Orthoptera	Acrididade	Sp.2	17.65 (45)	0
		Sp.3	19.61 (50)	3.21 (7)
		Sp.4	11.76 (30)	0
	Total		100 (255)	(100) 255

Table 2 Percentage of visits of florivorous consumed different floral structures (C-corolla, FA-feeding anthers, PA- pollinating anther and St-stigma) in two populations of *S. rostratum*. In parentheses the total number of visits made by florivorous

Florivorous	Amalucan				Cholula			
	C	FA	PA	ST	C	FA	PA	ST
Diabrotica undecimpuctata duodecimnotata	0	0	0	0	1.4 (3)	1.8 (4)	0	0
Macrodactylus mexicanus	4.7 (12)	1.6 (4)	2.0 (5)	0	0	0	0	
Centralaphthona sp.	2.7 (7)	0.4 (1)	0.4 (1)	0	17.4 (38)	3.2 (7)	0.5 (1)	0
Sp. 1	5.1 (13)	2.7 (7)	4.3 (11)	0.4 (1)	10.6 (23)	7.3 (16)	4.6 (10)	0
Epitrix sp.	23.9 (61)	1.2 (3)	1.2 (3)	0.4 (1)	36.7 (80)	5.0 (11)	7.3 (16)	0.9 (2)
Sp.2	14.5 (37)	2.7 (7)	0.4 (1)	0	0	0	0	0
Sp.3	15.7 (40)	3.5 (9)	0.4 (1)	0	3.2 (7)	0	0	0
Sp.4	9.8 (25)	1.6 (4)	0.4 (1)	0	0	0	0	0
Total	76.5 (195)	13.7 (35)	9.0 (23)	0.8 (2)	69.3 (151)	17.4 (38)	12.4 (27)	0.9 (2)

Table 3 Mean (± standard error) proportion, expressed as percentages, of each compound among the volatiles collected by GC-MS from the damage and undamaged flowers of *Solanum rostratum*

1 Mass spectra analysis and comparison of the normal alkane Retention Index with NIST library

2 Compared with synthetic compound

ID	Compounds	Retention time (min)	Retention index	Flowers	
				Undamaged	Damaged
1	Unknown 1	4.947	798	2.26 ± 1.93	4.64 ± 5.63
2	3-(Z)-Hexen-1-ol acetate [1]	6.279	918	3.09 ± 1.98	4.73 ± 6.42
3	Unknown 2	7.161	1071	7.72 ± 5.44	6.00 ± 4.51
4	Unknown 3	7.476	1112	3.91 ± 2.40	3.17 ± 2.04
5	Menthol [1,2]	8.349	1192	2.48 ± 1.58	5.28 ± 5.12
6	Unknown 4	8.571	1214	3.98 ± 1.74	3.19 ± 2.03
7	Tridecano	9.449	1301	1.55 ± 0.75	1.42 ± 1.34
8	2-Undecanol[1]	9.541	1311	1.52 ± 0.78	1.33 ± 1.13
9	(Z)-2-Tridecene[1]	9.616	1319	2.28 ± 1.16	1.45 ± 0.81
10	Eugenol[1,2]	10.164	1377	1.37 ± 0.45	1.60 ± 1.33
11	α-Copaene[1,2]	10.324	1394	19.96 ± 7.65	26.32 ± 23.08
12	α-Bourbonene[1]	10.420	1404	1.71 ± 1.22	3.13 ± 3.80
13	Methyleugenol[1,2]	10.483	1412	11.93 ± 5.25	5.49 ± 2.38
14	Cedrene[1]	10.753	1442	1.78 ± 0.63	3.88 ± 3.64
15	α-Farnesene[1,2]	10.792	1447	3.26 ± 1.64	2.50 ± 2.40
16	γ-Muurolene[1]	10.839	1452	2.23 ± 0.32	1.59 ± 0.51
17	γ-Decalactona[1,2]	11.140	1486	7.23 ± 1.78	4.82 ± 2.46
18	β-Cadinene[1]	11.207	1494	2.76 ± 0.53	2.21 ± 0.86
19	γ-Cadinene[1]	11.295	1504	3.25 ± 1.58	3.31 ± 3.33
20	δ-Cadinene[1]	11.570	1537	3.42 ± 0.78	2.68 ± 1.36
21	Hexadecano[1,2]	12.106	1601	1.42 ± 0.09	1.39 ± 0.54
22	β-Sinensal[1]	12.607	1665	4.81 ± 1.04	4.22 ± 1.59
23	Unknown 5	13.011	1718	2.24 ± 1.33	1.26 ± 0.73
24	Unknown 6	13.179	1740	1.83 ± 0.73	0.85 ± 0.45
25	1-Nonadecene[1]	14.288	1891	2.26 ± 1.25	2.13 ± 1.77

Table 4 Eigenvectors of the first two principal components (PC1 and PC2) of the principal component analysis of undamaged and damaged flowers of *Solanum rostratum*

ID	Compounds	PC1 (28%)	PC2 (22%)
1	Unknown 1	**-0.53962886**	0.23294656
2	3-(Z)-Hexen-1-ol acetate	0.38280051	-0.05869382
3	Unknown 2	**-0.59456309**	-0.3677267
4	Unknown 3	**-0.73341253**	-0.39666315
5	Menthol*	-0.34802975	0.36561441
6	Unknown 4	**-0.75759561**	-0.43462924
7	Tridecano	**-0.65039888**	-0.42624547
8	2-Undecanol	**-0.68328424**	-0.44372222
9	(Z)-2-Tridecene	**-0.77208228**	**-0.58178888**
10	Eugenol*	-0.41650436	0.29147647
11	α-Copaene*	**0.88747614**	**0.67006282**
12	α-Bourbonene	-0.26549237	-0.11328304
13	Methyleugenol*	-0.17275871	**-0.61133265**
14	Cedrene	-0.34749752	0.4550475
15	α-Farnesene*	0.49743587	-0.35461535
16	γ-Muurolene	-0.0338239	**-0.59280581**
17	γ-Decalactona*	-0.40702791	**-0.67175839**
18	β-Cadinene	-0.02894012	**-0.64432605**
19	γ-Cadinene	-0.15501295	**-0.53040757**
20	δ-Cadinene	-0.1633926	**-0.80529098**
21	Hexadecano*	**-0.56424747**	-0.08587569
22	β-Sinensal	**-0.61180975**	**-0.54618991**
23	Unknown 5	0.41111485	-0.26160516
24	Unknown 6	0.28091135	-0.37470596
25	1-Nonadecene	-0.42132353	-0.3304381

Table 5 Classification of legitimate and illegitimate visitors of *S. rostratum* flowers in Amalucan population

Proportions of legitimate visits during a visitor contacted only the stigma, both types of anthers and the stigma (FA, PA, ST), or one anther type (feeding or pollinating anther) and the stigma (FA/PA, ST) during all visits, including legitimate and illegitimate visits [if the visitor contacted only the feeding anthers (FA), the pollinating anther (PA), or both anthers types (FA, PA)].

Bee species	Sexual organ contacted during visit						Total visits contacting stigma and anthers	Total visits observed	Visit duration (s) ± S.E.	Proportion of legitimate visits
	Anthers and stigma		Only anthers			Only stigma				
	FA/PA, ST	FA, PA, ST	FA	PA	FA, PA	ST				
Legitimate visitors										
Bombus sonorus	1	78	23	1	17	0	79	120	4.64 ± 3.00	0.66
Xylocopa sp.	0	20	0	0	0	0	20	20	3.57 ± 1.59	1.00
Sub total	1	98	23	1	17	0	99	140		0.71
Illegitimate visitors										
Anthophora sp.	5	7	10	3	50	0	12	75	4.01 ± 1.80	0.16
Apis mellifera	6	6	10	9	11	1	12	43	4.06 ± 3.30	0.28
Augochlora sp.	0	1	9	10	3	0	1	23	6.78 ± 4.33	0.04
Augochloropsis metallica	0	0	1	2	0	0	0	3	8.75 ± 8.13	0
Lasioglossum dialictus	0	0	7	2	4	0	0	13	10.15 ± 8.54	0
Syrphidae	0	0	5	4	9	1	0	19	7.93 ± 5.05	0
Sub total	11	14	42	30	77	2	25	176		0.14
TOTAL	12	112	65	31	94	2	124	316		0.39

Fig. 1 Florivorous in two populations of *Solanum rostratum* [(a) Amalucan and (b) Cholula population] during observation periods

Fig. 2 Volatile compounds of PC2 in undamaged (C) and damaged (F) flowers of *S. rostratum*

Fig. 3 a) Number of visits and b) duration of visits conducted by floral visitors (legitimate and illegitimate) on undamaged flowers (Control) and damaged flowers manually (Manual) or by florivorous (Florivory) in bioassays in field conditions

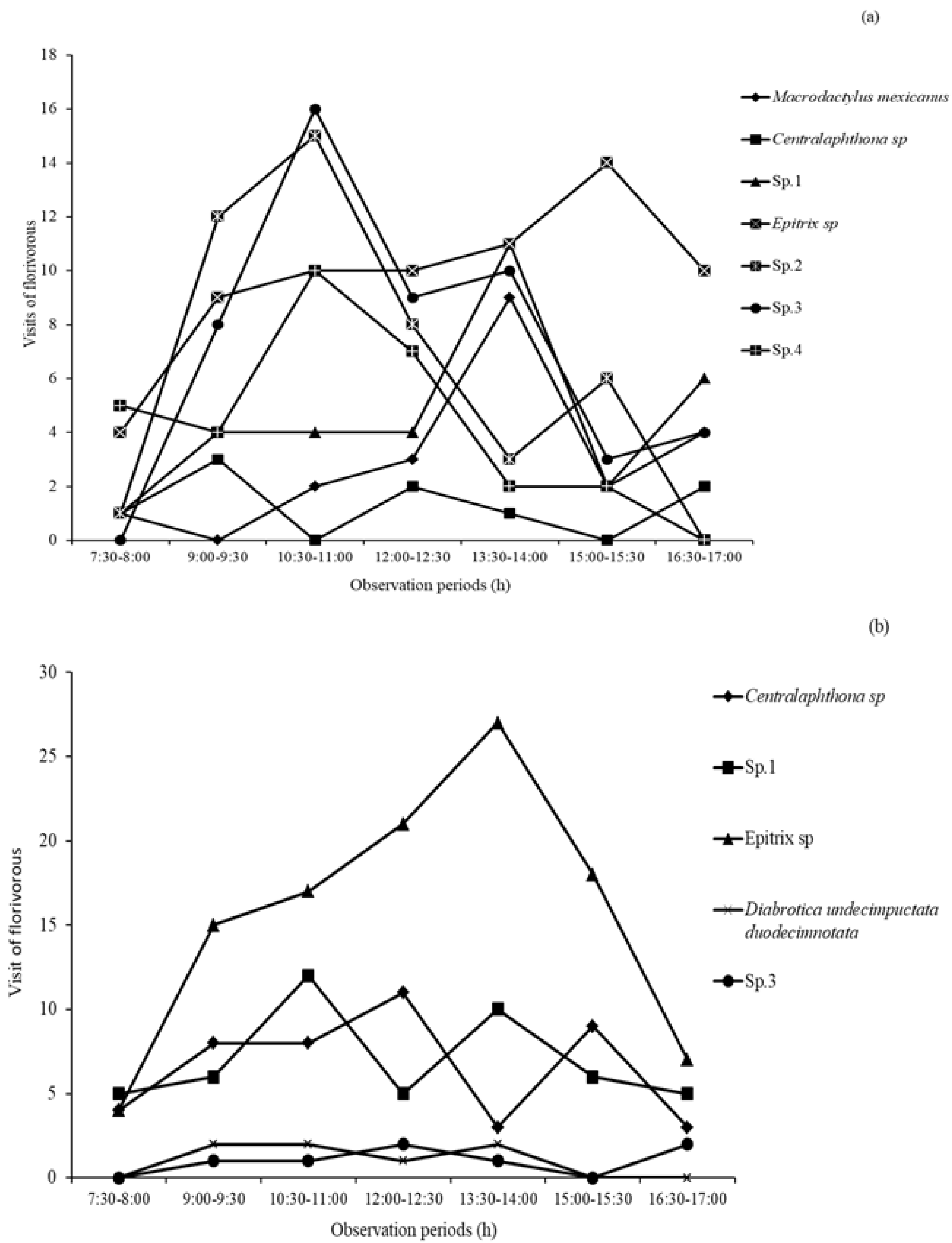
(a)
Visits of florivorous
Observation periods (h)
7:30-8:00
9:00-9:30
10:30-11:00
12:00-12:30
13:30-14:00
15:00-15:30
16:30-17:00
Macrodactylus mexicanus
Centralaphthona sp
Sp.1
Epitrix sp
Sp.2
Sp.3
Sp.4
(b)
Visit of florivorous
Observation periods (h)
Centralaphthona sp
Sp.1
Epitrix sp
Diabrotica undecimpuctata duodecimnotata
Sp.3

Figure 1

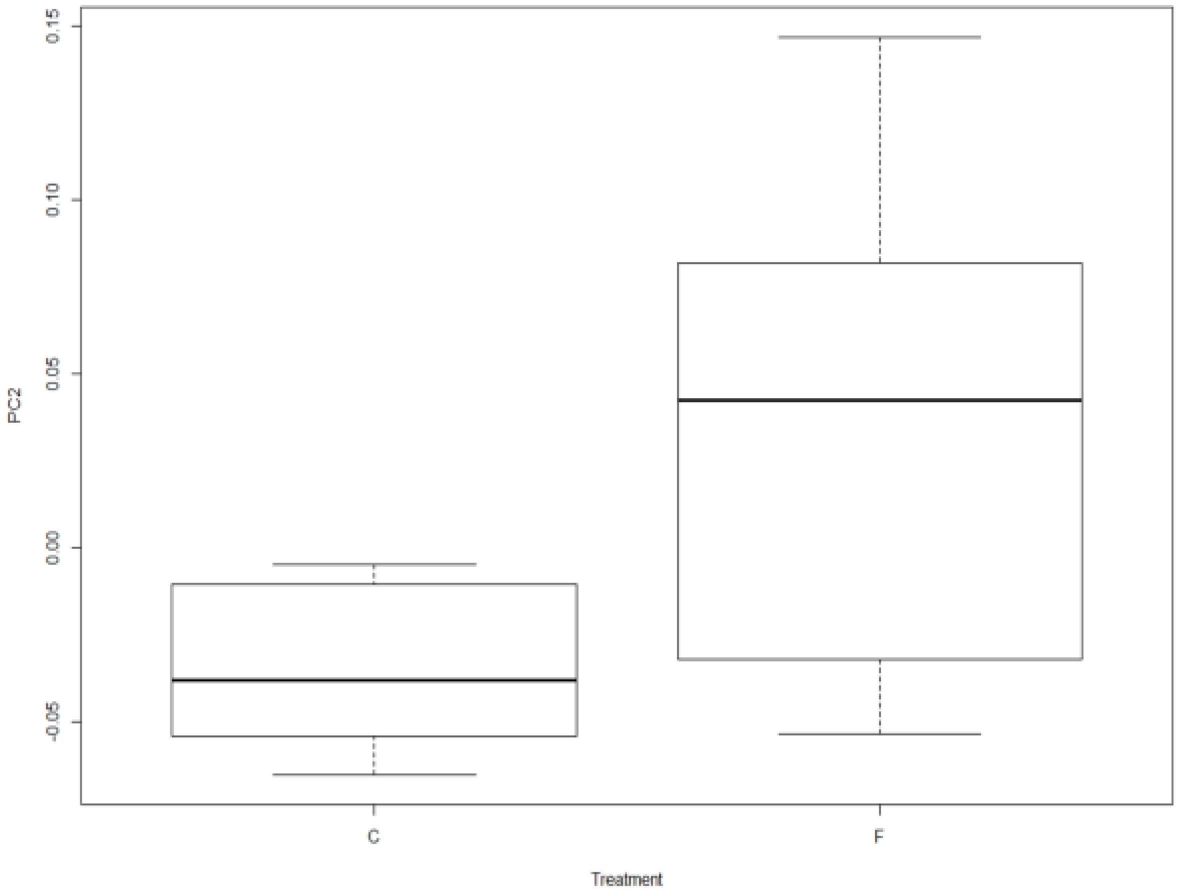

Figure 2

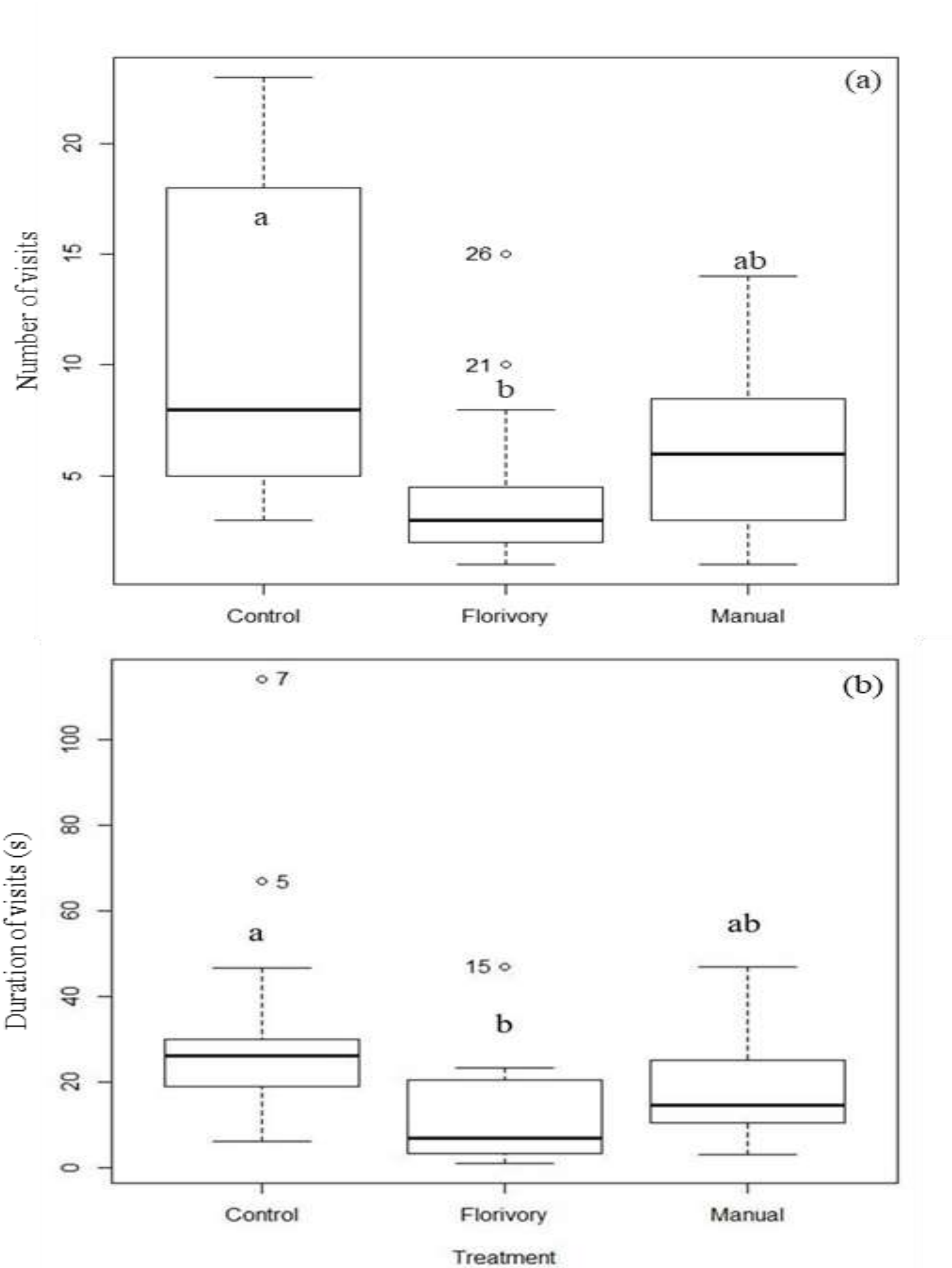
(a)
Number of visits
20
15
10
5
a
26
ab
21
b
Control
Florivory
Manual
(b)
Duration of visits (s)
100
80
60
40
20
0
7
5
a
ab
15
b
Control
Florivory
Manual
Treatment

Figure 3

Capítulo III. Conclusiones

La herbivoría floral implica el daño ocasionado por un animal a las estructuras reproductivas de las plantas, o a las estructuras de atracción a los polinizadores i.e., pétalos, sépalos, brácteas o lígulas. A pesar de que la florivoría puede ocasionar la destrucción de las flores, provocando una marcada disminución en la adecuación de las plantas; su repercusión sobre el desarrollo y el éxito reproductivo de las plantas ha sido poco estudiada (McNaughton 1983; Wallace and O'Dowd 1989; Lowenberg 1994; Cunningham 1995; Breadmore and Kirk 1998; McCall and Irwin 2006). De igual forma este tipo de estudios en plantas con morfología floral especializada son escaso, por lo que en esta investigación determinamos el impacto de los florívoros en el éxito reproductivo de *Solanum rostratum* una planta polinizada por vibración que depende fuertemente de sus polinizadores. A través de la caracterización de los principales florívoros de esta planta en dos poblaciones naturales; la preferencia de los individuos antagonistas por las estructuras reproductivas o de atracción para los polinizadores; con la determinación de los compuestos volátiles que se emiten por las flores dañadas por florívoros y flores sin daños. Además, evaluando cómo se afecta la atracción de los polinizadores y la producción de frutos y semillas.

Para las dos poblaciones estudiadas de *S. rostratum* encontramos ocho especies de florívoros. Representadas taxonómicamente por los órdenes Coleoptera y Orthoptera, y las familias Chrysomelidae, Scarabaeidae y Acrididae coincidiendo con el estudio desarrollado por Rodríguez-Islas (2016). El florívoro *Epitrix* sp. fue el que más flores dañó, resultando además el más abundante en ambas poblaciones.

La preferencia de los insectos antagonistas estuvo dada por más del 69 % de visitas donde consumieron corola, un 17 % donde consumieron las anteras alimenticias y en un 12 % la antera polinizadora.

En cuanto a la influencia del daño floral a la atracción de los polinizadores, consideramos dos tipos de visitantes, i.e., legítimos cuando contactaron ambos órganos sexuales (estigma y anteras) e ilegítimos cuando no contactaron dichas estructuras. Encontramos a *Bombus sonorus* y *Xylocopa* sp. Previamente reportados por Bowers (1975) y Solís-Montero et al. (2015) como polinizadores eficientes de esta planta debido a que son abejas grandes zumbadoras que contactan los órganos sexuales. Mientras que *Augochlora sp., Augocloropsis metallica, Lasioglossum dialictus* ya han sido reportadas previamente como ladronas de polen debido a que al ser abejas pequeñas sólo contactan las anteras para extraer el polen por vibración (Bowers 1975, Solís-Montero et al. 2015). Por su parte, Bowers (1975) ya había registrado *Anthophora,* una abeja grande que zumba, como visitante de *S. rostratum* en Estados Unidos. Sin embargo, en este estudio encontramos que sólo el 16% de sus visitas fueron legítimas por lo que podría estar funcionando como un ladrón condicional o polinizador poco eficiente. Al igual que *Apis mellifera* que a pesar de que no vibra para extraer el polen ya ha sido previamente reportada como un ladrón condicional de esta maleza (Solís-Montero et al. 2015). De manera general, en este estudio los visitantes florales (tanto ladrones como polinizadores) visitaron más las anteras alimenticias que la polinizadora como ha sido reportado para otras especies con especialización en las anteras probablemente a que las anteras alimenticias presentan fácil acceso y a las señales visuales y olfativas que son más atractivas para los visitantes florales (Li et al. 2015; Mesquita-Neto et al. 2017; Carvalho Velloso et al. 2018; Solís-Montero et al. 2018). De manera general, las flores dañadas fueron menos visitadas que las flores sin daño. Evidenciando que el daño en las estructuras de atracción o reproductivas afecta la visita de los polinizadores y que puede estar relacionado con que el deterioro de corola y anteras, así como en cambios en las proporciones del perfil de los compuestos volátiles que se emiten, influyendo en el comportamiento de los insectos mutualistas. Cuando se determinó los compuestos volátiles emitidos por flores con daño por florívoro y flores sin daño, encontramos 25 compuestos químicos, los cuales se presentaron en ambos tratamientos, pero en diferentes proporciones. Con presencia de α-copaene, methyleugenol, el compuesto desconocido 2 y γ-decalactona en mayores proporciones.

La producción de frutos y semillas no mostraron diferencias entre flores con diferentes porcentajes de daño debido a que los datos no fueron suficientes. Sin embargo, con esta investigación pretendemos aportar conocimientos novedosos al estudio de esta relación mutualista-planta-antagonista, específicamente en esta especie de morfología floral especializada. Se recomienda realizar en el futuro bioensayos con condiciones más controladas para poder evaluar si la disminución en el número de vistas a las flores dañadas repercute en la producción de frutos y semillas. Así como, con el propósito de contribuir al desarrollo de estudios posteriores de este tema sugerimos realizar bioensayos de preferencia de compuestos volátiles en diferentes proporciones tanto con florívoros como con polinizadores de *S. rostratum* para indagar el papel de estos compuestos en la atracción o repelencia de sus insectos visitantes.

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